Determination of perchlorate at parts-per-billion levels in plants by ion chromatography

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Abstract

A method for the analysis of perchlorate in plants was developed, based on dry weight, and applied to the analysis of plant organs, foodstuffs, and plant products. The method reduced greatly the ionic interferences in water extracts of plant materials. The high background conductivity, due to the plant matrix, was reduced sufficiently to allow quantitation of perchlorate with little or no matrix interference. Ion chromatography (IC) on a microbore AS16 anion exchange column and a conductivity detector was used for separation and detection of perchlorate from the ionic plant extract. The extract was heated to precipitate proteins, centrifuged, exposed to alumina, and filtered through a cartridge filled with divinylbenzene to yield a water clear extract for IC analysis, even from highly colored solutions. Heating the extract and treatment with alumina reduced substantially the ionic content of the extracts without loss of perchlorate.

1. Introduction

Perchlorate (ClO_4^-) is a contaminant in the environment that resulted from the use of the solid salts of ammonium, potassium, and sodium perchlorate in rockets, missiles, fireworks, and the manufacture of matches. The Interagency Perchlorate Steering Committee (IPSC) was formed to bring together representatives from the United States Environmental Protection Agency and Department of Defense and other government representatives and affected State, Tribal, and local governments to facilitate and coordinate technological issues related to perchlorate. The IPSC recently issued a report that assesses the state-of-the-science on the health effects of perchlorate on humans and the environment and the extent of perchlorate contamination [1]. Reported here is research conducted to develop a method for measuring the concentrations of perchlorate in foods and plants by ion chromatography (IC). Ion chromatography is a routine technique for the analysis of inorganic ions in plant organs and foods. IC methods are reported for halide anions and the oxygen species of nitrogen, sulfur, and phosphorus as well as several of the oxy anions of chlorine, bromine and iodine [2]. The most widespread detection technique used with IC is conductimetry coupled with post-column suppression of eluent ions. The evolution of self-regenerating suppressors that enhance analyte conductivity while suppressing eluent conductivity allows parts-per-billion (ppb) detection limits for anions and cations without preconcentration [3].

Perchlorate has a highly delocalized anionic charge and large atomic volume. The resulting low affinity to cations caused by the low charge density of the anion is the major

contributing factor to the high water solubility of perchlorate salts [4]. The AS16 column recently introduced by Dionex is a hydrophilic anion exchange column with a high capacity (170 µequiv./column) that allows the simultaneous analysis of large polarizable anions, such as iodide, thiosulfate, thiocyanate, and perchlorate. Also, the high capacity of the AS16 column allows quantitation of perchlorate at 5 ppb (µg/L) in the presence of up to 1000 mg/L (ppm) sulfate ion [5,6]. However, environmental matrices and other samples often have small organic molecules such as amino acids, sugars, fatty acids, and nucleotides in addition to a variable pool of inorganic ions which contribute to the ionic environment of the plant. Separation of perchlorate is affected by these non or weakly retained organic and inorganic ions to the extent the minor perchlorate peak is either not detected or must be quantitated as a shoulder peak.

Several methods are commonly used for preconcentration of analyte ions of interest. Trace enrichment of analyte ions has been accomplished by large volume injections of the sample on the analytical column. Also, precolumn sample enrichment is accomplished by pumping an accurately known volume of sample through a small ion exchange precolumn that is known to selectively retain the analytes of interest. The retained analytes are eluted onto an ion exchange analytical column for separation and quantitation [7]. Recently detection and quantitation of perchlorate by electrospray ionization mass spectrometry (ESI-MS) was described [8,9]. The method is based on the formation of a stable tetralkylammonium:perchlorate association complex that is extracted into an organic solvent. The complex is quantitated by ESI-MS with a lower limit of detection reported to be 10 ppb.

This manuscript describes the stepwise method that was developed to reduce the levels of interfering matrix ions in water extracts of plant organs and foodstuffs without a corresponding decrease in the level of perchlorate.

2. Experimental

2.1 Plant material and preparation

The lettuce and tomatoes used in the study were purchased from a local food chain. The lettuce was cored and the leaves cut into 1-2 cm pieces and freeze-dried. The skin was removed from the tomato fruit and the remaining pulp was cut into 1-2 cm cubes and freeze-dried. The pulp included all of the parenchyma tissue, locular tissue and gel, and the seeds. The tobacco leaf product was shredded before freeze-drying. All freeze-dried material was ground through a 30 mesh screen in a Wiley mill and stored in air-tight containers at 3°C until analyzed. Dry weights were obtained in duplicate by weighing 0.5g aliquots of the freeze-dried material and heating at 105°C for 24 hr. The percent moisture in the samples before freeze drying were 94% for lettuce and tomato fruit, and 81% for the tobacco leaf product.

2.2 Sorbent materials

2.2.1 Silica and Alumina Adsorbents

Unisil silicic acid (100-200 mesh) was obtained from Clarkson Chemical (Williamsport, PA). Silica gel (100-200 mesh) was Fisher reagent grade A.C.S. (Fairlawn, NJ). The Alcoa F-1 and DD-6 aluminas were a gift of Dr. Jacimaria Batista (UNLV, Las Vegas, NV). The DD-6

description: Al_2O_3 91.5%, surface area = 380 m^2/g, 8 x 14 U.S. screen mesh, was provided by Alcoa Port Allen Works (Port Allen, LA). The silicas and aluminas were cleaned by mixing one volume of solid with ten volumes of 18 MΩ water. The cloudy water (fines) above the solid material was decanted and replaced with fresh water until the water remained clear. The cleaned sorbents were air dried and stored in a screw cap container until used. The pH of 3 mL aqueous slurries of 1g samples of unwashed and washed sorbents were measured on an Orion EA®920 expandable ion analyzer (Cambridge, MA) after a 24 hr equilibration .

2.2.2 OnGuard® RP cartridges and Acrodisc® syringe filters

OnGuard® RP cartridges filled with a macroporous divinylbenzene reversed phase packing were obtained from Dionex (Sunnyvale, CA). Before application of the extract the cartridges were washed with 5 mL of methanol and then with 10 mL of 18 MΩ water at a flow rate of 3-4 mL/min. The Pall-Gelman Laboratory 0.2 µm ion chromatography Acrodisc® ion membrane syringe filters were obtained from Fisher Scientific (Fairlawn, NJ) and used as received.

2.3 Sample Preparation

Approximately 600 mg, in duplicate, of each freeze-dried sample were weighed and placed in 45-mL capacity centrifuge tubes. Thirty mL of water was added to half of the duplicates and 30 mL of 1000 ppb perchlorate was added to the remaining duplicates. All water used was at least 18 MΩ water. The centrifuge tubes, containing the samples, were tightly capped and placed in a boiling water bath for 0.5 hr to both precipitate protein and to saturate the dried material. The samples were placed in a 3°C refrigerator and shaken every 2 hr then allowed to set overnight. Total time of extraction was 20 hr. The samples were centrifuged at 20,000 x g for 30 min. The supernatants were gently poured through 1 layer of perchlorate free Kimwipes® and the mother liquor again centrifuged at 20,000 x g for 30 min. The pellet was discarded and the supernatant filtered through a 0.2 ∝m Acrodisc® filter. These colored solutions (yellow, green, and brown) were designated the "original extracts" when dilutions were made. Prior to analysis by IC, 1-mL aliquots of the original extracts were added to 500 mg of DD-6 alumina for 20 hr at 3°C, diluted 1:10, and filtered through in sequence a second 0.2 micron Acrodisc® filter, and a precleaned and activated OnGuard® RP cartridge. The first 0.75 mL was discarded and four 2-mL aliquots collected for IC analysis. The eluents were water clear.

2.4 Instrumentation

Ion chromatography was performed on a Dionex DX-500 system (Sunnyvale, CA). The chromatograph was equipped with a GP50 gradient pump, ED40 electrochemical detector, LC30 chromatography oven, and AS3500 autosampler. The ED40 was equipped with a conductivity cell and DS3 detection stabilizer maintained at 30°C within the LC30 oven. The conductivity cell was mounted at the end of the ASRS®ULTRA (2mm) self-regenerating suppressor that was operated at 100 mA and allowed detection of anions in the 0-10 µS conductivity range. Anions were separated on an AS16 analytical microbore separation column (2 x 250 mm) in tandem with

an AS16 guard column (2 x 50 mm). Samples and standards were run in the isocratic mode (0.38 mL/min) using 50 mM NaOH as eluent. Elution time of perchlorate varied from 9.84-11.7 min depending on the concentration of non-perchlorate ions and the extent of progressive column degradation. In all cases, a 1,000 µL injection loop was used. A 1,000 ppm stock solution of sodium perchlorate was prepared by weighing 1.231 g of sodium perchlorate into a 1L volumetric flask and bringing to volume with 18 MΩ water. Dilutions of the stock solution were made to span the range 5-200 ppb. Injections of these standards yielded calibration of the IC and measured concentrations estimated from the regression equation to be accurate within ≤1% of the true value. The correlation coefficient for regression of the averaged values was always greater than 0.999.

3. Results and Discussion

The objective of this research was to develop a method for the analysis of perchlorate at 500 ppb in foods and plant material; ideally the method would reduce the level of organic and inorganic ions that interfere with IC analyses without loss of perchlorate. Water extracts of plant materials contain both inorganic ions and organic ionic material. The water extract of the plant materials was heated in a boiling water bath for thirty minutes to precipitate the protein material that was subsequently removed by centrifugation and to breakdown cell walls and membranes to assure complete dissolution of perchlorate . The supernatant contained inorganic ions and the non-precipitated mostly lower molecular weight organic ions and colored pigments.

The fact that perchlorate is known to sorb weakly to most soil minerals, prompted the experiments with chromatographic adsorbents commonly used in the laboratory. Alumina was chosen over silicic acid for further investigation after preliminary experiments determined that the DD-6 and F-1 aluminas greatly reduced the ionic background in the plant extracts. The chromatograms before and after treatment with the silica sorbents were essentially the same. The ionic peak in the chromatogram of the DD-6 treated extract was narrower and returned to baseline sooner than in the F-1 treated extract. The DD-6 alumina was used in subsequent experiments. The pH of the aqueous layer above the solid sorbents was measured before and after washing. The pH of the aqueous layer of Fisher silica gel was 6.44 before and after washing while the pH of the Unisil silicic acid decreased from 6.55 to 6.07 after washing. The pH of the aqueous layer of the F-1 alumina decreased from 9. 83 before washing to 8.93 after washing. The pH decrease of the DD-6 aqueous layer was greater, 9.81 before washing and 7.22 after washing.

Alumina has three distinct surface site types for adsorption interactions: (a) acidic or positive sites, (b) basic or proton acceptor sites, and (c) electron acceptor (charge transfer) sites [10]. To determine sorption losses of perchlorate in the method, 1.0 mL of 1000 ppb perchlorate was added to 500 mg of DD-6 alumina, equilibrated for 20 hr, diluted 1:10 with 18 MΩ water, and the 100 ppb sample was filtered through in sequence a 0.2 µm Acrodisc® and an OnGuard® RP cartridge. Replicate experiments determined that the observed 60.8 ± 1.2 ppb (n = 3) recovery of perchlorate was attributable to sorption to alumina only. To test for possible competitive adsorption of other ions on the alumina, 1 mL of 1000 ppb perchlorate was added to 500 mg alumina together with 1 mL of a 1000 ppb solution of ammonium sulfate, ammonium

nitrate, sodium nitrate, and ammonium dihydrogen phosphate and equilibrated for 20 hr. These samples were diluted with 8 mL of water and passed through the Acrodisc® filter and OnGuard® RP cartridge. The recovery of perchlorate was 61.5 ± 1.9 ppb (n=4); the presence of other anions at the same 100 ppb concentration did not affect the sorption of perchlorate anion to alumina. The experiment was repeated but with 1000 ppm salt solution; recovery of perchlorate was 106.7 ± 1.1 ppb (n =4) and the broad IC ion peak, with perchlorate eluting as a shoulder peak, was similar to the plant extracts. In a separate experiment 50 ppb of perchlorate was added to 500 mg of a sandy soil and 35.6 ± 0.8 ppb (n = 3) was recovered. If 50 ppm salt solution was added with the perchlorate, recovery of the 50 ppb perchlorate spike was quantitative (53.9 ± 0.5 ppb n=4).

Raman spectroscopy is used for the analysis of both organic and inorganic compounds in aqueous media, but is hampered by a large background when the sample contains fluorescing components. The Raman spectra of the tomato extract, taken before and after exposure to DD-6 alumina, indicated a large reduction in the concentration of fluorescing compounds, some of which could be ionic, and an approximate threefold reduction in sulfate. To assess the selectivity and magnitude of the sorption of several common inorganic ions on DD-6 alumina, Raman analysis was performed on two aqueous solutions of inorganic ions before and after treatment of 1-mL aliquots with 500 mg of DD-6 alumina and dilution to 10 mL before Raman analysis. The first solution contained phosphate (1,000 ppm), sulfate (1,000 ppm), nitrate (2,000 ppm), and perchlorate (1,000 ppb) ions. After treatment with alumina, phosphate was not observed above the Raman limit of detection (≈30 ppm) in the 1:10 diluted sample and sulfate was reduced by approximately 70 % , while nitrate and perchlorate concentrations were unaffected. The experiment was repeated but phosphate was increased to 3,000 ppm. Again phosphate was not detected above the Raman limit of detection in the 1:10 diluted sample after exposure to alumina, but sulfate was only decreased approximately 25 %, while again nitrate and perchlorate concentrations were unaffected. A third experiment was conducted with nitrate (1,240 ppm) and perchlorate (125 ppm) only and without dilution of the treated sample. In this case nitrate was reduced by approximately 19 % after treatment with DD-6 alumina while perchlorate was reduced by approximately 15 %. This data suggest preferential sorption of the ions to DD-6 alumina in the order phosphate>>sulfate>nitrate≈perchlorate [11]. This is in agreement with the previously discussed 40 % sorption when aqueous perchlorate only was exposed to DD-6 alumina and competition with other ions was not a factor in its sorption.

The IC chromatograms of 1:10 dilutions of the water extracts of lettuce, tomato, and tobacco leaf had a broad ionic (matrix) peak such that perchlorate would be quantitated on a multipeak tailing baseline. In the lettuce extract the matrix peak width was reduced and minor peaks eliminated and recovery of 100 ppb added perchlorate was quantitative. Similar results were obtained with a tobacco leaf extract that contained endogenous perchlorate. Mean recovery of perchlorate from the spiked tobacco extract was 105 ± 3 ppb. Tomato extract was by far the "dirtiest" and contained several large peaks before and after the retention time window for perchlorate, but it was determined by spiking the tomato extract with perchlorate that the extract did not contain a detectable amount of perchlorate. A second tomato sample was extracted with 1000 ppb aqueous perchlorate and the extract diluted 1:10 to yield 100 ppb perchlorate. Figure 1 shows an overlay of two chromatograms of a 1:10 dilution of these extracts. Chromatogram A is the 1:10 dilution of the untreated water extract. Identification and quantitation of perchlorate

would be questionable due to the high ionic background and multitude of peaks. Chromatogram B was obtained after treatment of 1 mL of the 1000 ppb in perchlorate extract with 500 mg of alumina, diluting 1:10, and filtering through a 0.2 micron Acrodisc® filter. The interfering ions were greatly reduced and the perchlorate peak was quantitated at 100 ppb.

In subsequent analyses it was determined that filtering the DD-6 alumina treated plant extracts through OnGuard® RP columns removed any remaining color and quantitation of perchlorate was unaffected. The alumina and RP column removed all color from the extracts and a large portion of the hydrophobic compounds, organic ions, and inorganic ions that eventually deteriorate or "foul" the AS16 guard and analytical columns. The degradation of column performance can be tracked by noting a gradual decrease in the retention time of perchlorate. Column cleaning with 3M HCl in 80% acetonitrile will clean the column sufficiently to restore the perchlorate retention times to within one minute of the original column retention time.

Shown in Figure 2 are four IC chromatograms obtained from collection of On-Guard® RP cartridge eluent in four 2-mL aliquots after discarding the initial 0.75 mL. Perchlorate was quantitatively recovered in the first two fractions but the background interferences increased in each fraction and quantitation of perchlorate was affected by increased matrix peaks in aliquots three and four. Apparently, the RP column had a limited capacity to retain the ionic material remaining in the extract after treatment with alumina and this capacity was often exceeded in fractions 2-4. However, perchlorate was not retained by the OnGuard® RP cartridge and quantitation could be performed with confidence in the first two 2-mL aliquots at 26 ppb and 25 ppb, respectively. The levels in aliquots three and four were 32 ppb and 42 ppb, respectively, due to merging of perchlorate with the increasingly larger peak that eluted prior to perchlorate.

The method detection limit (MDL) was determined using the procedure outlined in EPA Method 314.0 [12] using the AS16 column. Seven replicate injections of a 5 ppb perchlorate standard were analyzed and the MDL was calculated according to the equation:

$$MDL = tS$$

where t = 3.14 and is the Student's t-value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom and S = standard deviation of the replicate analyses. An MDL = 0.5 ppb was calculated for the analysis of perchlorate. The average signal to noise ratio (S/N) was 58/1 for injection of the 5 ppb standard. The minimum reporting level (MRL) is defined as the minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. The MRL is usually established at an analyte concentration either greater than three times the MDL or at a concentration which would yield a response greater than a signal to noise ratio of five. An MDL of 0.53 ppb and MRL of 4 ppb is reported for reagent water in EPA Method 314.

To estimate the MRL in lettuce and tomato matrices, 600 mg of dried samples of lettuce and tomato were extracted with 30 mL of 100 ppb aqueous perchlorate (3 µg) . The IC chromatograms for the cleaned up extracts of tomato and lettuce were similar and the IC chromatograms are shown for the lettuce matrix in Figure 3. The bottom chromatogram (C) is a 1:10 dilution of the 100 ppb aqueous standard of perchlorate that was used in the 30 mL extractions and it represents 100 % recovery of perchlorate. The middle IC chromatogram (B)

was obtained after treatment of the extract with alumina, diluting 1:10, and filtering through the RP cartridge. The top chromatogram (A) is from injection of a 1:10 dilution of the original 30-mL extract. Recovery of perchlorate from the tomato matrix (10.5 ± 0.3 n=4) and lettuce matrix (10.3 ± 0.1 ppb n=4) at S/N ≈ 115/1 was 100% based on comparison to replicate injections of the 10 ppb standard (10.6 ± 0.2 ppb n=4). The perchlorate peak in the untreated 1:10 lettuce extract (A) was essentially obscured by the interfering matrix and would not integrate. Perchlorate (3 µg) contained in the 30 mL of extract is equivalent to 5 ppm in the 600 mg dry sample; using the percent moisture determined for lettuce and tomato (94 %) this amount of perchlorate would be 500 ppb on a wet weight basis in both lettuce and tomato. The 100 % recovery of perchlorate from the lettuce and tomato matrices together with the high S/N ratio of the sample chromatograms support setting the MRL for the method at 5 ppb of perchlorate in the extract. This amount of perchlorate in the extract is equivalent to 250 ppb in each matrix on a wet weight basis.

4.0 Summary

A method was developed for the analysis of perchlorate in plant tissue at 5 ppm on a dry weight basis that was applied to the analysis of perchlorate in lettuce and tomato at the 0.5 ppm level on a wet weight basis. The clean-up procedure had the added benefit of prolonging the useful life of the AS16 guard and analytical columns. The initial extraction step used heat to both precipitate protein, which was removed by centrifugation, and to breakdown cell walls and membranes to assure complete dissolution of perchlorate. The ionic composition of the extract was further reduced to a level that allowed quantitation of perchlorate at the 10 ppb level in the final dilution of the original extract. The reduction in ionic composition of the extract, without loss of perchlorate, was achieved by treatment of the extract with DD-6 alumina and filtration through OnGuard® RP cartridges. The MRL of 250 ppb could possibly be lowered by decreasing the liquid to solid ratios and by use of other sorbents with higher ionic capacity or that preferentially sorb other ions in the presence of perchlorate.

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DISCLAIMER

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA.

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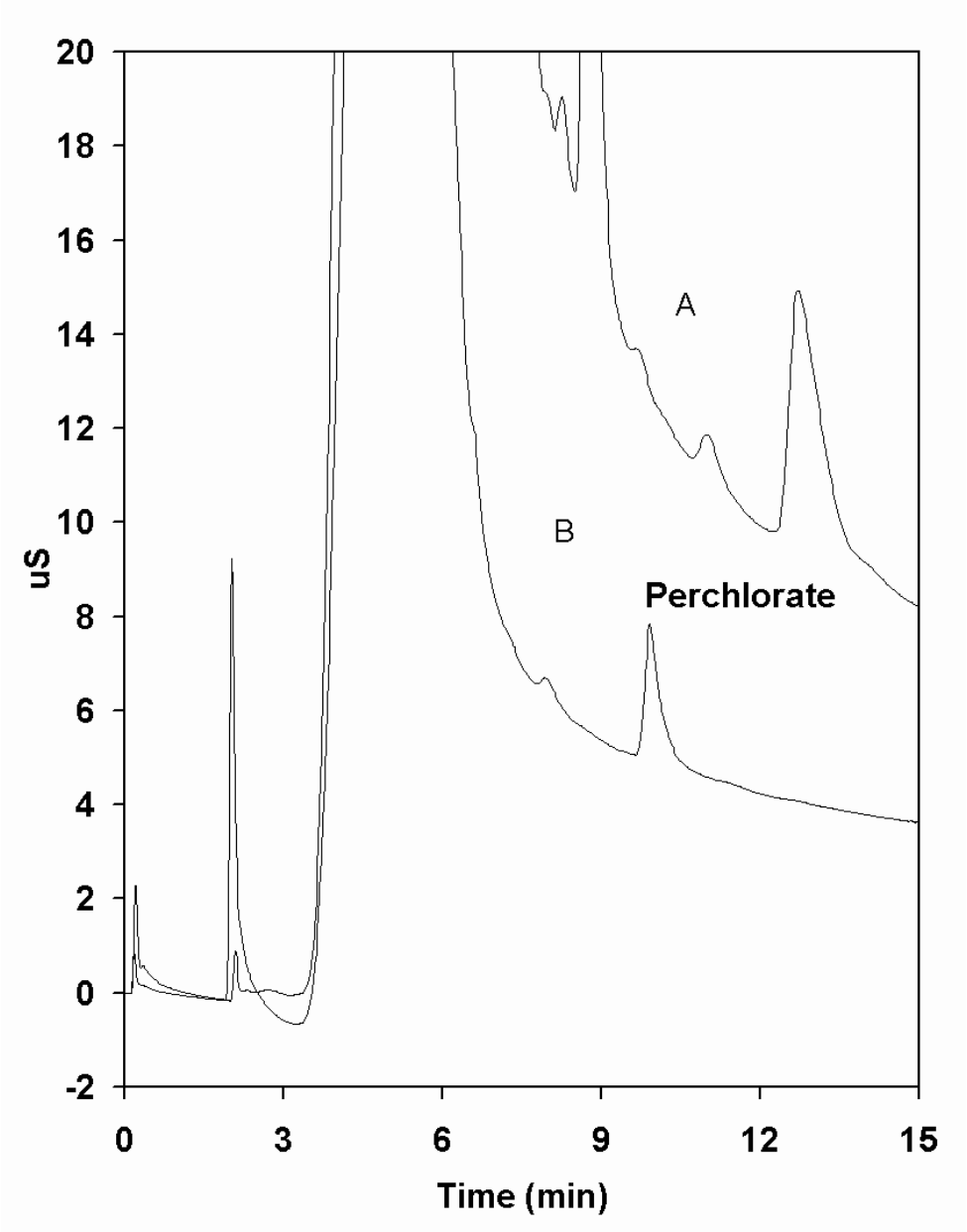

Fig. 1. Ion chromatograms showing the effect of DD-6 alumina on the matrix composition and perchlorate in tomato extracts. The upper chromatogram (A) is a 1:10 dilution of the original tomato extract with no alumina treatment. The lower chromatogram (B) is a 1:10 dilution of the original tomato extract after treatment with DD-6 alumina and fortified with 100 ppb perchlorate. See Experimental Section for extraction and chromatography conditions.

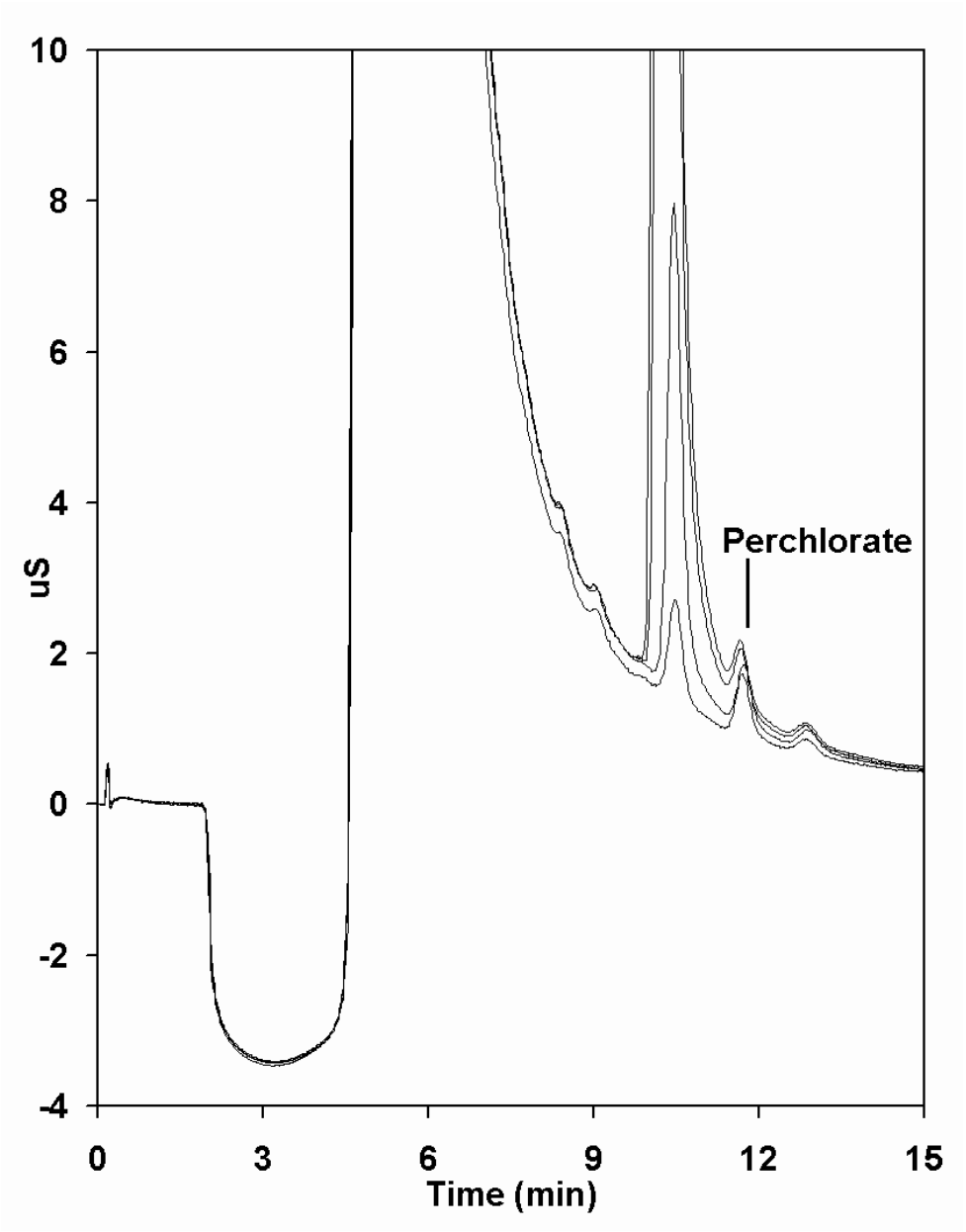

Fig. 2. Four superimposed ion chromatograms showing the effect of OnGuard® RP cartridges on the final clean-up of a tobacco leaf product extract after treatment with DD-6 alumina. Four, 2-mL fractions were collected after discarding the first 0.75 mL and run on the IC. The lowest plot represents the first 2-mL fraction and the second lowest plot represents the second 2-mL fraction. Fractions 3 and 4 are the upper 2 chromatograms and show the tobacco component going off scale and merging with the perchlorate peak.

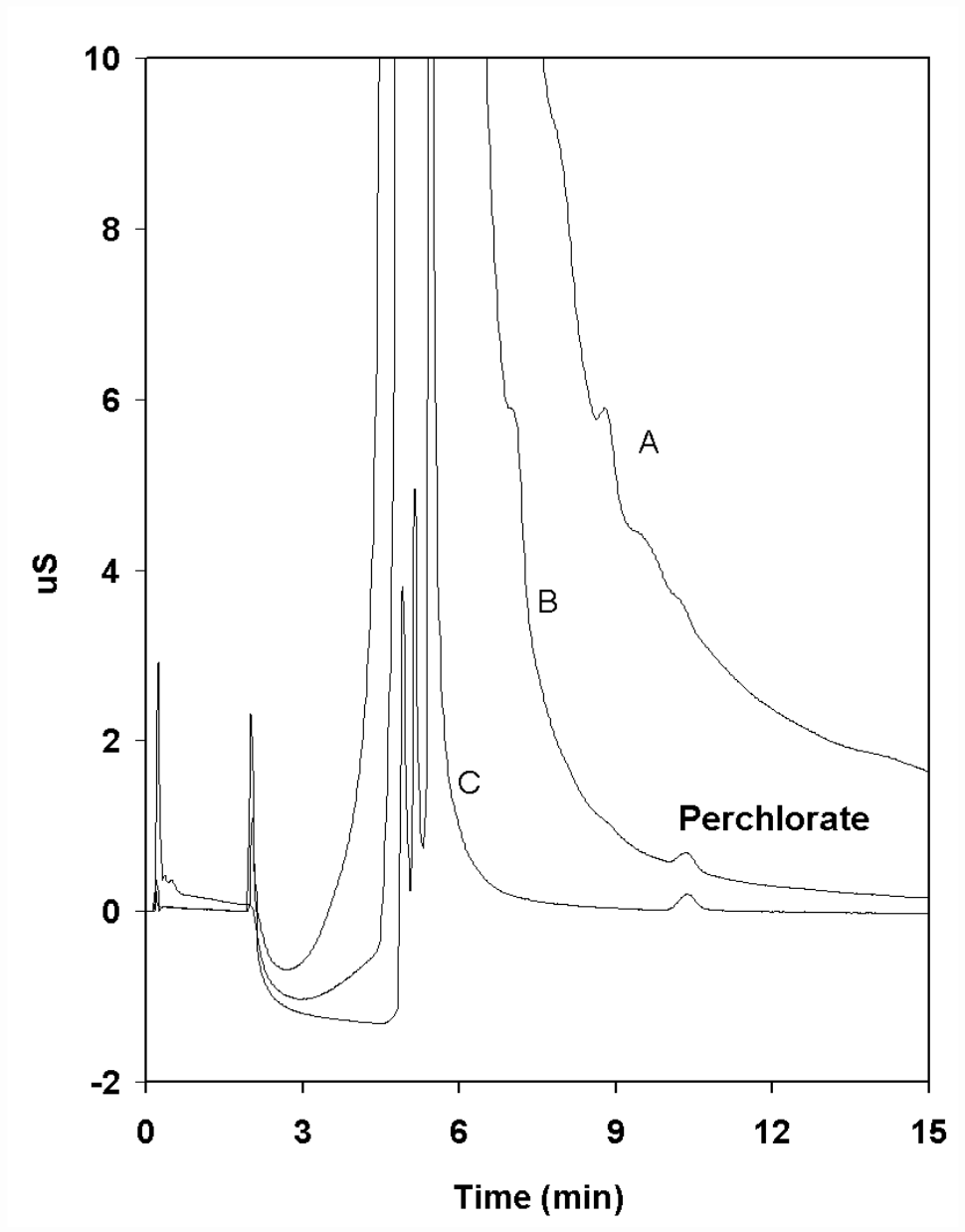

Fig. 3. Three overlaid chromatograms showing the elimination of much of the lettuce ionic matrix after treatment of a perchlorate containing extract of lettuce with DD-6 alumina and OnGuard® RP cartridges. The bottom plot (C) is a 10 ppb standard of perchlorate. The middle plot (B) is the cleaned up extract diluted 1:10. The top plot (A) is the original extract diluted 1:10.